

Golgi Bypass: Skirting Around the Heart of Classical Secretion

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Classical secretion consists of the delivery of transmembrane and soluble proteins to the plasma membrane and the extracellular medium, respectively, and is mediated by the organelles of the secretory pathway, the Endoplasmic Reticulum (ER), the ER exit sites, and the Golgi, as described by the Nobel Prize winner George Palade (Palade 1975). At the center of this transport route, the Golgi stack has a major role in modifying, processing, sorting, and dispatching newly synthesized proteins to their final destinations. More recently, however, it has become clear that an increasing number of transmembrane proteins reach the plasma membrane unconventionally, either by exiting the ER in non-COPII vesicles or by bypassing the Golgi. Here, we discuss the evidence for Golgi bypass and the possible physiological benefits of it. Intriguingly, at least during *Drosophila* development, Golgi bypass seems to be mediated by a Golgi protein, dGRASP, which is found ectopically localized to the plasma membrane.

The secretion of signal peptide-containing and transmembrane proteins through the cellular organelles that form the secretory pathway has been very well characterized over the years (Rothman 1994; Lee et al. 2004). During their translation, signal peptide-containing proteins are specifically recognized in the cytoplasm by the signal recognition particle and localize to the ER by virtue of the SRP binding its receptor (Nagai et al. 2003; Osborne et al. 2005). Other transmembrane proteins are embedded in the ER membrane by a

posttranslational mechanism called C-tail anchoring by the GET complex (Schuldiner et al. 2008). Following transfer into or across the ER membrane, nascent proteins undergo folding, oligomerization, and addition of oligosaccharide chains followed by exit via specialized landmarks, known as ER exit sites (ERES) in mammalian cells and transitional ER (tER) sites in yeast and *Drosophila*. Both sites are characterized by the presence of cargo-containing coat protein complex II (COPII)-coated vesicles (Bonifacino and Glick 2004; Lee et al. 2004). Thereafter, most proteins are transported through the Golgi (in a manner that is still very much debated) before reaching their final destination, such as the plasma membrane for

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many transmembrane proteins and the extracellular medium for secreted proteins (Mellman and Warren 2000) (Fig. 1, red arrows).

More recently, however, several examples of protein trafficking that deviate from this dogma

have been discovered. First, an increasing number of cytoplasmic proteins (such as IL-1 β , FGF2, MIF, and AcbA/Acb1) that do not harbor a signal peptide are found in the extracellular medium, and these display a wide range of

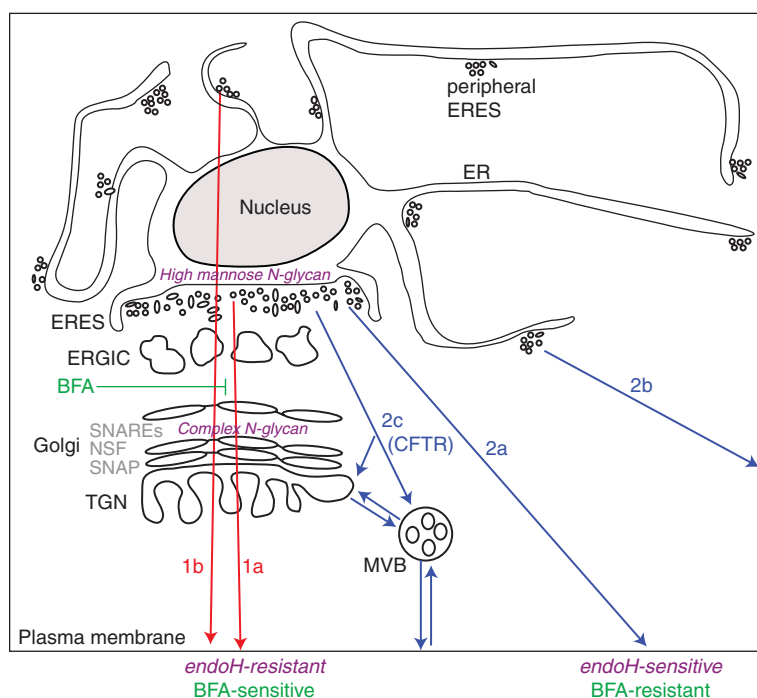


Figure 1. Classical trafficking, from the ER to the Golgi to the plasma membrane, is represented by the red arrows. A cargo protein can exit from an ERES in close proximity to the *cis*-Golgi (route 1a) or a peripheral ERES (route 1b), but irrespective of its ER exit, this protein follows a distinct pathway through the Golgi to the plasma membrane. This pathway is dependent on known SNARE proteins, NSF and SNAPs. As proteins pass from the ER and through the Golgi, their ER-derived high mannose oligosaccharides are modified by addition of complex sugars rendering these proteins EndoH-resistant. BFA treatment or loss of function of intra-Golgi SNAREs would lead to the retention of these proteins in the ER or Golgi and their diminished presence at the plasma membrane.

Potential routes for Golgi bypass are represented by blue arrows. Like classical cargo proteins, Golgi bypass cargoes may exit from an ERES near the *cis*-Golgi (routes 2a,c) or a peripheral ERES (route 2b). However, the immediate fate of these proteins deviates from the classical pathway. A protein following route 2a (from an ERES near the *cis*-Golgi) or 2b (from a peripheral ERES) would traffic on ER-derived transport intermediates directly to the plasma membrane, routes perhaps taken by CD45 or α PS1. This route would require a specific set of SNAREs, yet to be identified. As these proteins do not pass through the Golgi stack, their high mannose *N*-glycans remain sensitive to EndoH. These pathways are also revealed by blocking passage through the Golgi either by the application of BFA, or by the loss of function of intra-Golgi SNAREs, (e.g., Syntaxin 5), and observing their continued transport to the plasma membrane. Proteins that follow route 2c would bypass the Golgi stack via an endosomal intermediate, which would facilitate their delivery to the plasma membrane via conventional endosomal fusion machinery. In the case of CFTR, its exit from the ER may occur from either ERES location to the TGN or endosomes. If it is directly delivered to endosomes, it is likely recycled back to the TGN in which the observed oligosaccharide modifications take place before reaching the plasma membrane.



critical activities. This “cytoplasmic protein unconventional secretion” has been extensively discussed elsewhere (Nickel and Seedorf 2008; Nickel and Rabouille 2009) and will not be covered in this volume, except for a brief note toward the end. Second, a small subset of proteins does not exit the ER by virtue of classical COPII-coated vesicles. Third, a few transmembrane proteins have been shown to reach the plasma membrane, bypassing the Golgi, which is the focus of this article.

Why some proteins follow an unconventional route of secretion is intriguing but on the whole largely unknown. Through evolution, the cell has segregated processes within membrane compartments to maintain and optimize cellular functions. Why would mechanisms evolve to traffic a subset of proteins via unconventional routes? In this article, we discuss examples of Golgi bypass as well as outline why and how some proteins escape the conventional secretory pathway.

ATYPICAL ER EXIT VIA COPII-INDEPENDENT VESICLES

ER exit represents the first stage at which a transmembrane protein can deviate from the classical secretion route. Proteins destined for the plasma membrane exit via ERES and traffic to the ER-Golgi Intermediate Compartment (ERGIC) via COPII-coated vesicles. This process has been very well characterized. The large multidomain protein Sec16 is thought to provide a platform for ERES formation, which in combination with the Sec12 GEF leads to the activation and concentration of the Sar1 GTPase (Watson et al. 2006; Ivan et al. 2008; Hughes et al. 2009). The membrane recruitment of Sar1 initiates the sequential assembly of two coat heterodimers, Sec23-Sec24 and Sec13-Sec31, to complete the COPII coat (Gurkan et al. 2006; Stagg et al. 2006; Budnik and Stephens 2009). Recruitment of the COPII subunits, which are conserved in all eukaryotes, not only builds the coat structure, but also serves as a sorting device either directly or through a receptor for cargo selection (Stagg et al. 2008).

Hsp150, a soluble yeast glycoprotein, is an exception to this process. It contains an amino-terminal signal peptide and should, therefore, traffic conventionally to the plasma membrane. Its surface localization, however, is independent of the key COPII-coat components Sec13 and Sec24 (Fatal et al. 2004). Furthermore, ER exit of Hsp150-positive vesicles occurs at specific tER sites that do not contain Sec24. Whether Hsp150 also bypasses the Golgi is not yet clear and warrants further investigation.

A similar non-COPII mediated exit has been reported for the voltage-sensitive potassium channels, Kv4 K⁺. When associated with their interacting proteins, KChIPs, Kv4 K⁺ channel trafficking is unaffected by overexpression of dominant-negative Sar1 (Hasdemir et al. 2005), which is known to block COPII-mediated ER exit (Aridor et al. 1995; Rowe et al. 1996).

Last, the ER exit of EDEM1 (ER degradation-enhancing mannosidase-like 1) and OS-9 (an ER lectin) also provide an exciting example of cargo bypassing the COPII machinery. EDEM1 and OS-9 are key regulators of ER-associated degradation (ERAD) and target terminally misfolded glycoproteins in the ER for degradation by removing their mannose groups, thus accelerating their recognition by the ERAD machinery (Olivari et al. 2006; Christianson et al. 2008). To attenuate the ERAD activity in the ER under normal situations, EDEM1 and OS-9 are prevented from accumulating in this organelle and are rapidly delivered to late endosomes/lysosomes where they are turned over (Cali et al. 2008). Strikingly, their ER exit is mediated by a mechanism that seems to be COPII-independent (Zuber et al. 2007; Cali et al. 2008), and occurs at sites in the rough ER, distinct from typical ERES. This suggests that ER exit of these two proteins, like Hsp150, is spatially segregated along ER cisternae (Zuber et al. 2007; Cali et al. 2008). EDEM1 exit from the ER involves the protein LC3-I but not LC3-II, the lipid bound form of the protein that is a key marker of autophagy (Klionsky et al. 2008), indicating that EDEM1 exit is not mediated by the autophagic process (Reggiori et al. 2010). Furthermore, it is thought that this pool of EDEM1 en route to the endosomal system also bypasses the Golgi.

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DETECTING GOLGI BYPASS

EndoH Sensitivity

As mentioned in the introduction, following exit from the ER, most newly synthesized proteins reach the Golgi (Watson and Stephens 2005) in which they can be modified, proteolytically processed and dispatched to their final destination, the plasma membrane, the extracellular medium or an organelle of the endolysosomal system (with the exception of the mitochondria and peroxisomes) (Keller and Simons 1997).

One major protein modification that occurs in the Golgi, at least in mammalian cells, is construction of N-linked complex oligosaccharide chains (see Stanley 2011). The majority of proteins that enter the ER are glycosylated with a high mannose-containing, N-linked oligosaccharide core. After reaching the Golgi, oligosaccharide-modifying enzymes trim and add sugars to this core, generating complex N-glycans (Roth 2002) (Fig. 1; route 1a,b). Therefore, a protein that bypasses the Golgi would retain its initial high mannose oligosaccharide core. These changes in glycosylation status can be detected using Endoglycosidase H (EndoH).¹ One of the first modifications that occur in the Golgi is the addition of an N-acetylglucosamine sugar to the mannose oligosaccharide. The resulting product is no longer a substrate for EndoH (EndoH-resistant) (Medzihradzky 2005). Consequently, proteins that are resistant to EndoH are thought to be conventionally trafficked through the Golgi whereas those that remain sensitive to its activity could, in principle, have bypassed it. Therefore, EndoH sensitivity can be used as a read-out to detect Golgi bypass of proteins, however it does not define the pathway.

Brefeldin A Resistance

Transport through the early secretory pathway relies on another set of coated vesicular intermediates known as coatamer protein complex

¹EndoH catalyses the hydrolysis of the diacetylchitobiose core of the oligosaccharide between the two N-acetylglucosamine residues

I (COPI)-coated vesicles (see Lorente-Rodriguez and Barlowe 2011; Wieland 2011). The COPI coat consists of seven proteins that form a multiprotein complex, much like the COPII coat (Lee and Goldberg 2010), which, together with the small GTPase ARF1 decorates vesicular intermediates primarily formed at the Golgi. COPI-coated vesicles mediate retrograde movement of resident Golgi enzymes and proteins that have to be reused within the Golgi and also recycled back for ER exit (such as SNARE proteins and the KDEL receptor) (Pelham and Rothman 2000; Rabouille and Klumperman 2005). The ARF1 GTPase is essential for COPI coat formation (see Wieland 2011) and can be inhibited by Brefeldin A (BFA, see Box 1), a fungal toxin that interferes with recruitment to Golgi membranes and activation of ARF1 (Helms and Rothman 1992). BFA treatment thus blocks retrograde transport from the Golgi resulting in a strong impairment of anterograde trafficking through this organelle, and cargo delivery to post-Golgi compartments of the secretory pathway can be totally blocked. In most cells, BFA treatment also results in the absorption of the Golgi membrane into the ER (Lippincott-Schwartz et al. 1989). Therefore, it is assumed that a protein reaching the plasma membrane following BFA treatment does not use the conventional ER-Golgi secretory route. However, as discussed in Box 1, the Golgi in some cell types, such as polarized MDCK and PtK1 cells, is not reabsorbed into the ER after treatment with standard doses of BFA (Ktistakis et al. 1991; Chen et al. 1992; Oda et al. 1995). Even in these cells, however, BFA efficiently blocks the majority of conventional anterograde transport to the plasma membrane, albeit at much higher doses.

Golgi SNARE Independence

The fusion of vesicular intermediates in the secretory pathway is determined by SNAREs (soluble N-ethylmaleimide-sensitive fusion protein (NSF) accessory protein (SNAP) receptors) and SM (Sec1/Munc-18-like) proteins. These serve to specifically bring two membranes into close proximity to drive lipid bilayer fusion

(see Malsam and Söllner 2011; Sudhof and Rothman 2009). SNAREs can be divided into two classes: target SNAREs (t-SNAREs) and vesicle SNAREs (v-SNAREs), which are present exclusively on apposing heterotypic membranes. One such t-SNARE, Syntaxin 5 (or Sed5 in yeast) is required for transport to and through the Golgi (Dascher et al. 1994; Nichols and Pelham 1998; Rowe et al. 1998). The importance of Syntaxin 5 in classical secretion is emphasized by the observation that its loss in

all model organisms blocks classical secretion through the Golgi (Hardwick and Pelham 1992; Amessou et al. 2007; Schotman et al. 2008).

To summarize, combinations of BFA treatment (and the resulting inhibition of ARF GEFs; see Box 1), transport in the absence of Golgi SNAREs and careful monitoring of the glycosylation state of cargo proteins are often used to identify proteins that might bypass the Golgi.

BOX 1. What does Brefeldin A do?

The fungal toxin Brefeldin A (BFA) acts on a subset of Sec7 domain-containing GTP-exchange factors (GEFs) responsible mainly for the activation of ARF1 GTPase, a protein essential to the formation of COPI coats on donor membranes in the secretory pathway (as discussed in the main text). Treatment with BFA interferes with the GDP-GTP exchange by binding to the initial complex between ARF1, GDP, and the GEFs (such as GBF1, BIG1, and BIG2), thus preventing the exchange and release of activated ARF1-GTP. As a result, the formation (and localization) of COPI coats on Golgi membranes is inhibited.

In most cells (most commonly used being HeLa or COS-7 cells), the loss of COPI coats from Golgi membranes leads to the formation of extensive tubules from the Golgi along microtubule networks (within 5–10 min of BFA application), which do not detach, but are eventually absorbed into the ER (Sciaky et al. 1997). BFA treatment essentially leads to the formation of a hybrid ER-Golgi compartment, which can be described as a morphological phenotype. As a result, the use of BFA causes a block in bulk flow from the ER to and through the Golgi, a functional effect of BFA.

Some cell lines, however, have been reported to be “BFA-resistant” because treatment with this toxin does not cause the morphological phenotype of reabsorption of the Golgi into the ER. The most characterized BFA-resistant cells are MDCK. It has recently been shown that MDCK cells express a point mutant version of the ARF-GEF, GBF1, which makes these cells resistant to BFA (Lanke et al. 2009). Although this remains to be proven, it is possible that the mutation interferes with BFA binding to GBF1. In support of this interpretation, BFA-treated polarized MDCK cells do not lose their COPI coat from the Golgi and a hybrid ER-Golgi compartment does not form. Nevertheless, in polarized MDCK cells, ER to Golgi transport to the apical plasma membrane is inhibited, whereas transport to the basolateral side is unaffected.

However, when MDCKs are grown under conditions that do not sustain polarity (i.e., in a low calcium and low temperature environment), BFA leads to the reabsorption of the Golgi into the ER (exactly as HeLa cells), perhaps suggesting that the GBF1 mutation is thermo-sensitive and calcium-sensitive. Furthermore, recent work has shown that treating polarized MDCK cells with 5- to 50-fold higher doses of BFA is sufficient to block bulk apical and basolateral transport through the Golgi (Tveit et al. 2009). This observation suggests that the point mutation in GBF1 may lead to its desensitization to BFA, which can be overcome simply by increasing the drug concentration. Essentially, polarized MDCK cells display a functional sensitivity to BFA treatment that is seemingly uncoupled from the ‘known’ morphological effect of the drug on the Golgi, at least at low doses. Interestingly, it has been shown that high doses of BFA induce Golgi bypass in MDCK cells (discussed further in the main text). Taken together, although the use of BFA as an inhibitor of ER-Golgi protein trafficking in the secretory pathway has become a common tool, there are differences, sometimes subtle, in its effects dependent on cell type and BFA concentration used. Furthermore, BFA also affects the TGN, endosomes, lysosomes, and plasma membrane, suggesting the existence of additional molecular targets.

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A summary of these effects can be seen in the table below:

Table 1. Effects of BFA on different cell types.

Cell type	Concentration	Collapse of Golgi into the ER	Block of ER to Golgi transport?	Other effects?	References
HeLa CHO Cos-7 NRK HepG2 Rat Hepatocytes	1–10 µg/ml	Yes	Yes	-Lysosomes form a microtubule-dependent network -Tubulated TGN connects with endosomes (mixed TGN/EE)	(Misumi et al. 1986; Fujiwara et al. 1988; Doms et al. 1989; Lippincott-Schwartz et al. 1989; Lippincott-Schwartz et al. 1991; Pelham 1991; Wood et al. 1991; Strous et al. 1993; van Meer and van 't Hof 1993; Sciaky et al. 1997)
PtK1	2–5 µg/ml	No	No	- Tubulated early endosomes	(Ktistakis et al. 1991)
MDCK	1–5 µg/ml	No	-Yes, transport to the apical membrane -No, transport to the basolateral membrane is unaffected	-Selective inhibition of transcytosis -Enhanced TfR transcytosis -Tubulated endosomes -TGN tubulation -Stimulates apical transport and slows basolateral recycling of TfR -Increases apical endocytosis -Induces Golgi bypass in the apical direction	(Hunziker et al. 1991; Lippincott-Schwartz et al. 1991; Low et al. 1991; Pelham 1991; Sandvig et al. 1991; Prydz et al. 1992; Wan et al. 1992; Matter et al. 1993; Wagner et al. 1994; Futter et al. 1998; Shitara et al. 1998; Wang et al. 2001; Tveit et al. 2009)
Nonpolarized MDCK	2–5 µg/ml, low calcium and low temperature	Yes	Not tested		(Burdett 2002)
Polarized MDCK	50 µg/ml	Not tested	Yes, to apical and basolateral membranes	Induces Golgi bypass in apical and basolateral directions	(Tveit et al. 2009)
Caco-2	1–5 µg/ml	Yes	Yes	-Enhanced TfR transcytosis	(van Meer and van 't Hof 1993; Shah and Shen 1994; Bose et al. 1998)
<i>Drosophila</i> S2 cells	15–30 µg/ml	No	Yes	Clumping of the Golgi	(Xu et al. 2002a; Xu et al. 2002b; Kondylis and Rabouille 2003; Kondylis et al. 2007)

KNOWN CARGO BYPASSING THE GOLGI

To date, a small but significant cohort of transmembrane proteins able to traffic to their desired cellular sites in a BFA-resistant manner has been identified, suggesting that their transport does not require passage through the Golgi (Fig. 1; route 2a,b). Below, we describe evidence for a few of these examples.

CD45

A pool of CD45, a protein tyrosine phosphatase essential for T-cell and thymocyte development, has been shown to appear at the plasma membrane in a BFA-insensitive manner (Baldwin and Ostergaard 2002). In untreated T cells, CD45 reaches the plasma membrane in two differentially glycosylated forms, one EndoH-resistant (classical) and one EndoH-sensitive that potentially bypasses the Golgi. Interestingly, the pool of EndoH-sensitive proteins reaches the plasma membrane approximately three times faster than the EndoH-resistant forms, indicating that at least this Golgi bypass pathway supports faster secretion than the classical route. Furthermore, EndoH-sensitive CD45 appears on the cell surface under normal growth conditions, suggesting that Golgi bypass of CD45 might also be a constitutive process. Surprisingly, no tests have been made to assess whether the high mannose form of CD45 retains full phosphatase activity or the same substrate specificity. If the enzymatic activity of CD45 is altered, Golgi bypass could represent an interesting means by which to regulate CD45 activity.

Hemichannel Proteins

The transmembrane proteins of the connexin family form gap junctions. These are membrane channels connecting the cytoplasm of neighboring cells to allow the passage of solutes, ions, and signaling molecules (Kumar and Gilula 1996; Saez et al. 2003). Each connexin-mediated gap junction comprises two hemichannels each contributed by a neighboring cell, which are formed by six homo- or hetero-connexin dimers. Interestingly, some connexins (e.g., Connexin 26 and 30) are able to traffic in a BFA-resistant

manner, whereas others, such as Connexin 32 and 43 seem to be wholly conventional in their transport to the plasma membrane (Martin et al. 2001; Qu et al. 2009).

Although similar to connexins structurally (presence of 4 transmembrane segments and formation of membrane channels), pannexins have been shown to be evolutionarily distinct and are more homologous to innexins, the sole gap junction proteins that exist in invertebrates, (Baranova et al. 2004). Both innexins and pannexins form single intramembrane channels that communicate with the extracellular environment by allowing the passage of molecules and ions such as ATP and calcium ions, respectively (Bauer et al. 2005; D'Hondt et al. 2009). Like connexins, pannexins and innexins also homo- and hetero-oligomerize, and some of the subunits such as pannexin 1 and 3, are able to traffic both conventionally as well as reach the plasma membrane during BFA treatment, suggesting an interesting interplay between classical secretion and Golgi bypass (Penuela et al. 2007). Each of the human pannexins 1, 2, and 3 is uniquely glycosylated with a single oligosaccharide chain and their glycosylation status has been shown to regulate the mixing of the different subunits (Penuela et al. 2009). This observation provides an attractive possibility that Golgi bypass could regulate pannexin intermixing.

Drosophila α PS1 Integrin

Golgi bypass has also been observed in *Drosophila* follicular epithelial cells (Schotman et al. 2008; Schotman et al. 2009). During a key stage termed 10B in the development and growth of the egg chamber during oogenesis, the columnar epithelial cells that surround the oocyte are required to flatten to maintain their coverage. During this flattening process, new plasma membrane contacts with the extracellular matrix (ECM) are made by converting the basolateral membrane into basal contacts. The basal contacts with the ECM are generated by the use of adhesive proteins such as α PS1 integrin, the secretion of which sharply increases at this stage of *Drosophila* development. Surprisingly, this α PS1 secretion bypasses the Golgi because the

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transport of this integrin is BFA-insensitive and Syntaxin 5 independent. Before Stage 10B, interestingly, α PS1 secretion occurs conventionally, in a BFA-sensitive, Syntaxin-5-dependent manner.

CFTR

The most documented example of a cargo bypassing the Golgi stack is the cystic fibrosis transmembrane conductance regulator (CFTR), the protein mutated in the genetic disease cystic fibrosis (Tsui 1992; Welsh and Smith 1993). CFTR exit from the ER is dependent on COPII-coat components in all cell types (Wang et al. 2004). Astonishingly, in a cell-type specific manner and despite canonical ER exit, maturation of CFTR N-glycans into complex oligosaccharides is insensitive to the loss of ARF1, Syntaxin 5, and Rab1A/Rab2 GTPases, proteins required for transport from the ER to the Golgi (Yoo et al. 2002). Interestingly, CFTR transport through this unconventional route is blocked by overexpression of dominant negative Syntaxin 13, a t-SNARE that resides at the TGN/endosomal system. This result confirms the potential existence of a secretion route for CFTR from the ER to the plasma membrane passing through the TGN or endosomes, then on to the plasma membrane (Yoo et al. 2002) (Fig. 1; route 2c). As the N-glycans of CFTR are modified, it suggests that if CFTR reaches endosomes directly, it must be recycled to the Golgi prior to plasma membrane delivery.

Recently, the Golgi bypass of CFTR en route to the plasma membrane has implicated a pericentriolar intermediate compartment (pIC), positive for the small ER to Golgi GTPase Rab1a (Tisdale et al. 1992; Nuoffer et al. 1994), stably associated with the centrosome and with direct connections with the endosomal system (Santerud et al. 2006; Marie et al. 2009). Through the use of temperature shifts, a common method to block protein traffic in different compartments of the secretory pathway, CFTR has been shown to accumulate in the pIC. Following release of this block, CFTR is rapidly transported to the plasma membrane. Importantly, the morphology of the pIC and its association with recycling endosomes are

resistant to BFA treatment. However, in these cells, BFA blocked CFTR in the ER, but following washout of the drug, CFTR arrived rapidly at the pIC and the plasma membrane preceding Golgi reformation.

Induction of Golgi Bypass by BFA

Evidence has been gathered that Golgi bypass could be triggered not only during development, as described above for α PS1 integrins, but also pharmacologically, for instance following BFA treatment.

A small pool of the EndoH-sensitive proteoglycan Serglycin has been shown to arrive at the plasma membrane of polarized MDCK cells (Tveit et al. 2009). This pool, however, increases following BFA treatment. At low BFA concentration, EndoH-sensitive Serglycin reaches apical plasma membranes and at higher BFA concentrations, it is also delivered to basolateral membranes (see Box 1 for details). Under these conditions, Serglycin seems to be retained within the Golgi, leading to the hypothesis that cargo accumulation in this organelle may induce its bypass. This was tested further by artificially increasing the steady state level of Serglycin in the Golgi. Addition of KDEL to Serglycin (thus increasing its residency time in the early secretory pathway) in the absence of BFA also induced the delivery of an EndoH-sensitive pool to the apical plasma membrane (thus phenocopying BFA treatment). However, the effect of Serglycin-KDEL on the general secretory competence of the Golgi and the conventional traffic of other proteins was untested. Further work is needed to investigate whether cargo load in the Golgi influences the traffic mode.

WHY BYPASS THE GOLGI?

The cargoes that so far have been shown to bypass the Golgi are proteins of diverse functions, from channels to enzymes, to junction and adhesive proteins. Although the purpose of Golgi bypass is not well understood, potential reasons why a protein might traffic in this way can be identified.

Protection of Classical Cargo Proteins

Golgi bypass could be seen as a preventive measure that the cell employs to avoid deleterious effects on its secreted cargo. For instance, EDEM1 (the ER degradation-enhancing mannosidase-like 1, mentioned above) is thought to be delivered from the ER directly to endosomes/lysosomes without passing through the Golgi (Fatal et al. 2004; Zuber et al. 2007). One reason might be to ensure that EDEM1 mannosidase activity is diverted away from the main secretory route in which a multitude of glycosylated proteins reside or pass through, to possibly avoid the alteration of their oligosaccharides (Hosokawa et al. 2010).

Speed

Golgi bypass might also appear to be a faster process than classical trafficking through the Golgi, as observed with CD45 (Baldwin and Ostergaard 2002). Except for cells in which rapid secretion occurs (for instance, those of the [neuro]endocrine systems in which secretory vesicles are stored just beneath the plasma membrane ready to release following triggering), this enhanced speed of secretion may represent an efficient/fast way for the cell to up-regulate cell surface expression of a protein in response to extracellular stimuli. This might also be a way for highly polarized cells, such as neurons, to deposit membrane proteins to their growing tips (perhaps from a pool of localized mRNAs (Mohr and Richter 2003) that could be translated on the surrounding ER (Hanus and Ehlers 2008)), without relying on the secretory apparatus of the cell body (including the Golgi), sometimes located meters away. A pathway similar to this is presented in Figure 1 (route 2b).

Regulation of Protein Activity

Golgi bypass could also be employed to modulate the activity of proteins. The immature, EndoH-sensitive form of CD45 on T cells could specifically be recognized by mannose binding lectins, which are found, for instance, on the surface of stromal cells (Baldwin and

Ostergaard 2001). Vital to T-cell function is the ability to migrate to sites of cell-to-cell adhesion and of inflammation or injury (in which mannose binding lectins have key roles), both processes required for the activation of key signaling cascades. It is possible that Golgi bypass of CD45 supports specific stronger adhesion events between T cells and mannose binding lectins.

Alternatively, Golgi bypass may regulate the phosphatase activity and/or specificity of CD45. Although not related to CD45, receptor tyrosine phosphatase β (RPTP β) activity and signaling is inhibited by glycosylation (Abbott et al. 2008), suggesting that the enzymatic activity of protein phosphatases can be sensitive to glycosylation.

Although the reason why *Drosophila* α PS1 might bypass the Golgi of follicular epithelial cells is unknown, glycosylation and truncation modifications that occur in the Golgi have been shown to modulate the adhesive properties of integrins (Guo et al. 2003; Chen et al. 2006). As the oocyte expands, the follicular epithelium flattens, necessitating a modulated adhesion event through integrins showing differential biochemical properties. In addition, at approximately the same developmental time-point (stage 10B of oogenesis) that α PS1 is unconventionally secreted, α PS2 starts being expressed and synthesized (Delon and Brown 2009). It is known that α integrin subunits are chaperoned by the β subunits and they leave the ER as a dimer. It is therefore conceivable that α PS1 takes an alternative secretory route (together with a yet unknown chaperone) to allow the formation of the α PS2/ β PS dimer, which is thus then conventionally deposited on the surface as a functional complex after stage 10B.

Ensuring Correct Function of the Golgi

The major function of CFTR is to transport chloride ions across membranes (Akabas 2000) and this seems to be crucial for the maintenance of the pH gradient across Golgi membranes. This, in turn, plays a key role in the modification and secretion of proteins. Therefore, the

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presence of chloride in the cytoplasm, and the function of chloride channels as well as of lysosomal/vacuolar ATPases are critical for the maintenance of the Golgi intra-luminal pH and in turn of its functional integrity (Edwards and Kahl 2010). In this regard, one likely possibility is that CFTR is routed away from the early Golgi to maintain its pH gradient and secretory competence. Whether CFTR displays differential activity or is more functional in the Golgi rather than the ER is unknown.

Connexins and pannexins also form channels through the plasma membrane of mammalian cells, which allow passage of small molecules and ions (Kumar and Gilula 1996; Saez et al. 2003). Although the site at which different connexin and pannexin monomers multimerize and become functional is much debated and is often dependent on the subtype (for example, there is evidence of oligomerization of connexins in the ER, ERGIC, and TGN (Koval 2006)), the Golgi bypass of a number of subunits could help prevent the formation of gap junction channels in the Golgi, a process that could also prove to be detrimental to the function of the organelle.

BYPASSING THE GOLGI: HOW? A ROLE FOR GRASP

The molecular mechanism sustaining conventional secretion is exquisitely complex and tightly regulated. How might the Golgi bypass pathway overcome this rigid regulation? Perhaps the ERES used for this alternative trafficking segregates cargo bypassing the Golgi away from classical anterograde transport. COPII vesicles carrying Golgi bypass cargo might contain a different set of SNAREs thus allowing their direct fusion with the plasma membrane (i.e., Fig. 1; route 2a). Therefore, Golgi bypass is likely to rely on unknown combinations of SNAREs or on specific molecules that are yet to be identified.

However, what is known about Golgi bypass suggests that it uses molecules originally identified for their role in classical trafficking, but in an unusual way. For instance, the ER exit of EDEM1 depends on LC3, a key component and

marker of autophagy (Klionsky et al. 2008). However, EDEM1 exit depends on a nonlipidated form of LC3, which is thought to have no direct role in autophagy itself (Reggiori et al. 2010).

Recently, in the process of understanding the mechanism of α PS1 unconventional transport in *Drosophila* (see “Known Cargo Bypassing the Golgi”), the protein dGRASP was found to be a critical component of this alternative pathway. dGRASP is the single *Drosophila* homolog of mammalian GRASP55 and GRASP65. To date, these two proteins have been localized only to the Golgi and they play a role in Golgi organization, as stacking factors (Barr et al. 1997; Shorter et al. 1999; Xiang and Wang 2010), and mediators of Golgi ribbon formation (Puthenveedu et al. 2006; reviewed in Ramirez and Lowe 2009; Vinke et al. 2011). Surprisingly, at the stage of α PS1 unconventional deposition to the basal membrane, dGRASP was also found anchored at the same membrane. Furthermore, the transport of α PS1 integrin was strictly dependent on dGRASP, as, in a null allele of *dgrasp* it was retained intracellularly, whereas the classical anterograde transport of other transmembrane proteins through the Golgi complex remained unaltered (Schotman et al. 2008). Interestingly, the ectopic localization of dGRASP is not because of a repositioning of the Golgi or to its trafficking from the Golgi to the plasma membrane, but to a specific translation of its targeted mRNA and its subsequent local anchoring to the plasma membrane. The up-regulation and localization of *dgrasp* mRNA is triggered by developmental events such as mechanical pressure generated on the epithelium by the increasing size of the oocyte that augments epithelial tension (Schotman et al. 2009).

Interestingly, other mRNAs encoding proteins involved in the vesicular transport in the early compartments of the secretory pathway such as Sec16, Rab6, NSF1, SNAP, and Gos28 have been shown to localize to the same plasma membrane domains as *dgrasp* transcripts. Because these potential translation products are components of the early compartments of the secretory pathway, it has been proposed that

their localization to the plasma membrane at key developmental stages would allow the tethering of the ER-derived carriers to the cell periphery

Strikingly, in both *Dictyostelium* and yeast, GRASP has also been shown to be involved in the unconventional secretion of the cytoplasmic protein AcbA/Acb1 (Kinseth et al. 2007; Duran et al. 2010; Manjithaya et al. 2010). If GRASP also plays a role in tethering membrane intermediates involved in this secretion, then the last steps of unconventional secretion of AcbA/ACb1 and Golgi bypass of transmembrane proteins may bear similarities (Nickel and Rabouille 2009).

CONCLUDING REMARKS AND PERSPECTIVES

Many questions remain regarding Golgi bypass and more direct evidence of its existence is required. The core molecular components that regulate this pathway are largely unknown with the exceptions of Syntaxin 13 and dGRASP. It remains to be seen whether these proteins work within the same pathway, or whether they act within separate Golgi bypass routes. Testing the requirement of each of these proteins in the trafficking of cargoes such as CD45, CFTR, and α PS1 in their respective systems would aid our understanding of Golgi bypass. Furthermore, how these proteins might function to regulate Golgi bypass is largely unknown.

A major unanswered question is whether the GRASP-mediated Golgi bypass pathway observed in *Drosophila* operates in other epithelia and/or species and what other cargoes it may traffic. In *Drosophila* follicular epithelial cells, this pathway is under strong developmental control. It is possible that the GRASP-mediated Golgi bypass pathway has been missed previously because this developmental control element is missing in tissue culture environments commonly used for trafficking studies. If this were true, then developing methods that allow this pathway to be revealed in mammalian tissue culture would help in unraveling the molecular requirements, cargoes, and mechanisms of Golgi bypass. Furthermore, live-cell imaging

of a protein taking a direct route from the ER to the plasma membrane is still elusive. With a greater understanding of the machinery that drives Golgi bypass, we may one day observe a protein “skirting around the Golgi” in real time.

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Golgi Bypass: Skirting Around the Heart of Classical Secretion

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